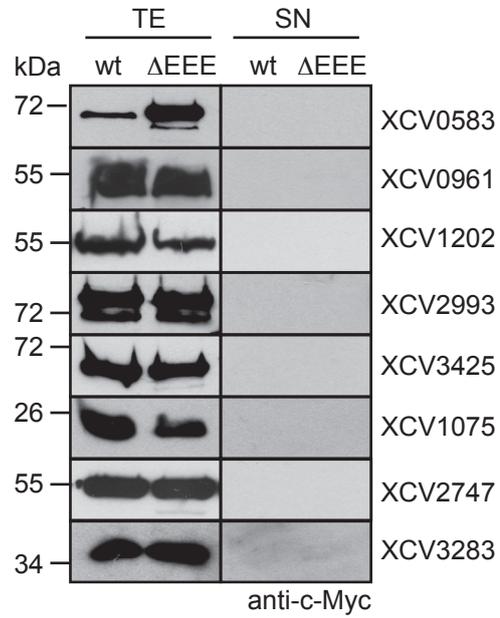
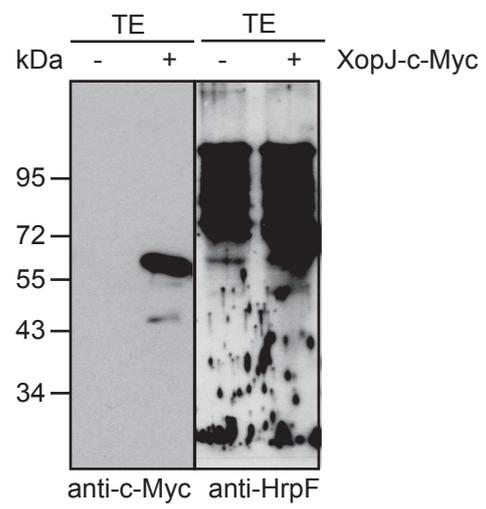


A



B



C

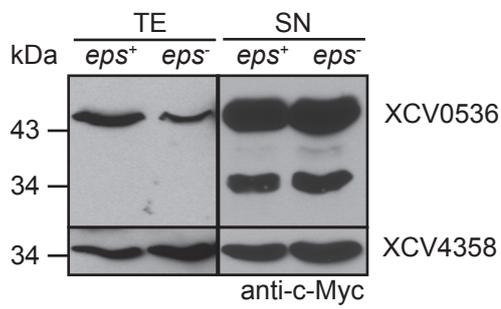


Fig. S1 Secretion assays with candidate T2S substrates from *Xcv*.

(A) Strains 85-10 (wt) and 85-10 Δ EEE (Δ EEE) carrying expression constructs encoding C-terminally c-Myc epitope-tagged candidate T2S substrates as indicated were incubated in NYG medium. Total cell extracts (TE) and culture supernatants (SN) were analysed by immunoblotting using a c-Myc epitope-specific antibody. To detect XCV0961-c-Myc, bacteria were incubated over night in NYG medium.

(B) The c-Myc epitope-specific antibody does not detect unspecific proteins in cell extracts of *Xcv*. Strain 85* with (+) and without (-) an expression construct encoding XopJ-c-Myc was incubated in minimal medium and cell extracts were analysed by immunoblotting using c-Myc epitope- and HrpF-specific antibodies. HrpF is the putative translocon protein of the T3S system that is synthesized in strain 85*.

(C) Secretion of XCV0536 and XCV4358 is not affected by EPS. Strains 85-10 and 85-10 ϵ ps encoding XCV0536-c-Myc and XCV4358-c-Myc as indicated were incubated in NYG medium. TE and SN were analysed as is described in panel A.

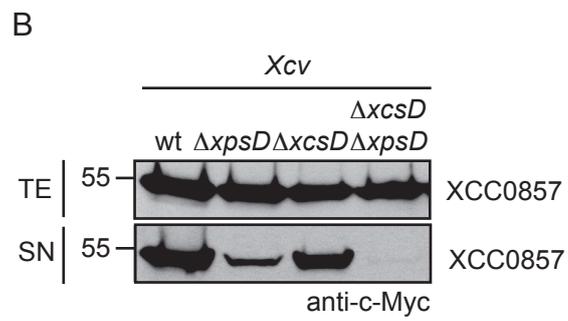
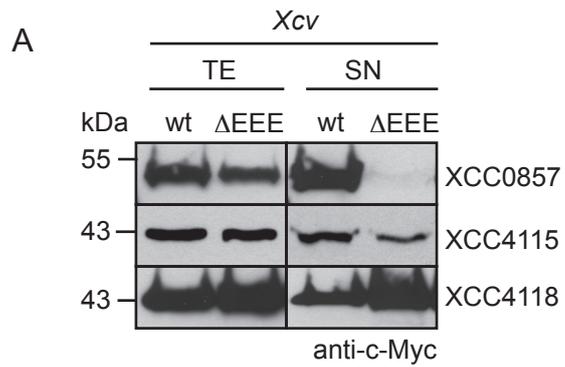
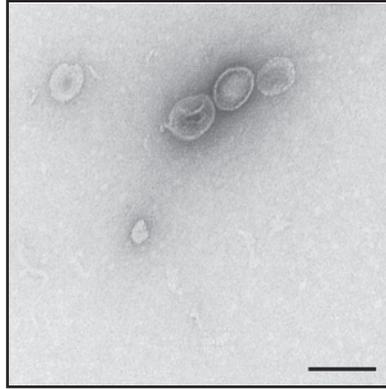


Fig. S2 XCC0857 is secreted by the Xps-T2S system of *Xcv*.

(A) The T2S systems of *Xcv* contribute to the secretion of XCC0857 but not of XCC4115 and XCC4118. *Xcv* strains 85-10 (wt) and 85-10 Δ EEE (Δ EEE) carrying XCC0857-c-Myc, XCC4115-c-Myc or XCC4118-c-Myc as indicated were incubated in NYG medium. Total cell extracts (TE) and culture supernatants (SN) were analysed by immunoblotting using a c-Myc epitope-specific antibody.

(B) Secretion of XCC0857 in *Xcv* depends on *xpsD*. *Xcv* strains 85-10 (wt), 85-10 Δ *xpsD* (Δ *xpsD*), 85-10 Δ *xcsD* (Δ *xcsD*) and 85-10 Δ *xcsD* Δ *xpsD* (Δ *xcsD* Δ *xpsD*) carrying XCC0857-c-Myc were incubated in NYG medium. TE and SN were analysed as is described in panel A.

A



B

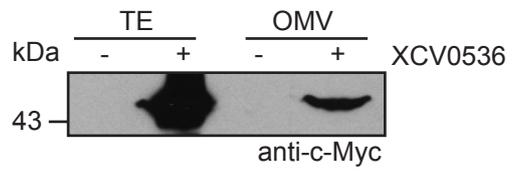


Fig. S3 Isolated OMVs from *Xcv* strain 85-10 expressing XCV0536-c-Myc.

(A) *Xcv* strain 85-10 containing XCV0536-c-Myc was incubated in NYG medium. Isolated OMVs were inspected by electron microscopy. The size bar corresponds to 100 nm.

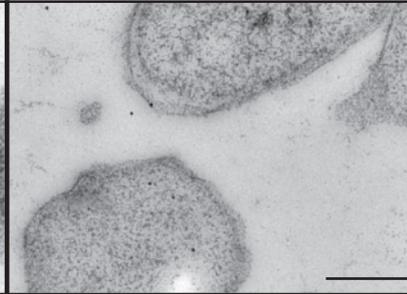
(B) Immunoblot analysis of isolated OMVs. Bacterial cell extracts and isolated OMVs were analysed by immunoblot analysis using a c-Myc epitope-specific antibody. TE, cell extract; OMV, isolated OMVs.

85-10

EV

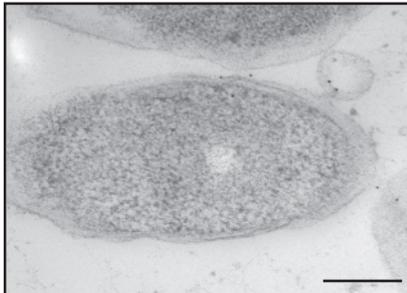


EV

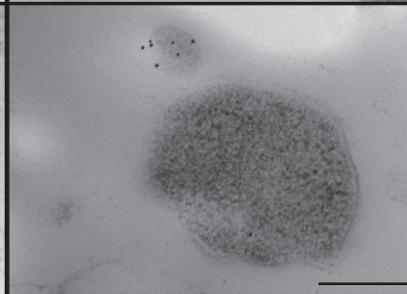
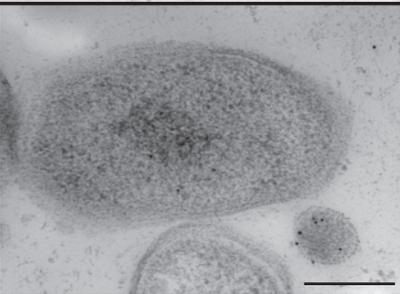
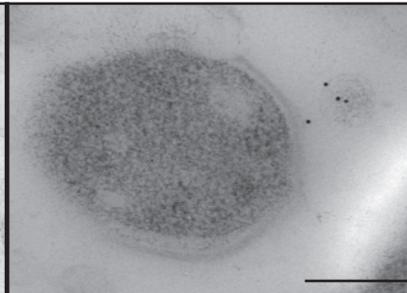


85-10 Δ EEE

XCV0536



XCV3671



XCV4358

XCV4360

Fig. S4 OMV formation in strain 85-10 and 85-10 Δ EEE. *Xcv* strains 85-10 and 85-10 Δ EEE containing vector pBRM (EV) or expression constructs encoding XCV0536-c-Myc, XCV3671-c-Myc, XCV4358-c-Myc or XCV4360-c-Myc as indicated were analysed by immunoelectron microscopy using a c-Myc epitope-specific antibody and a secondary antibody coupled to gold particles. The size bar corresponds to 250 nm.

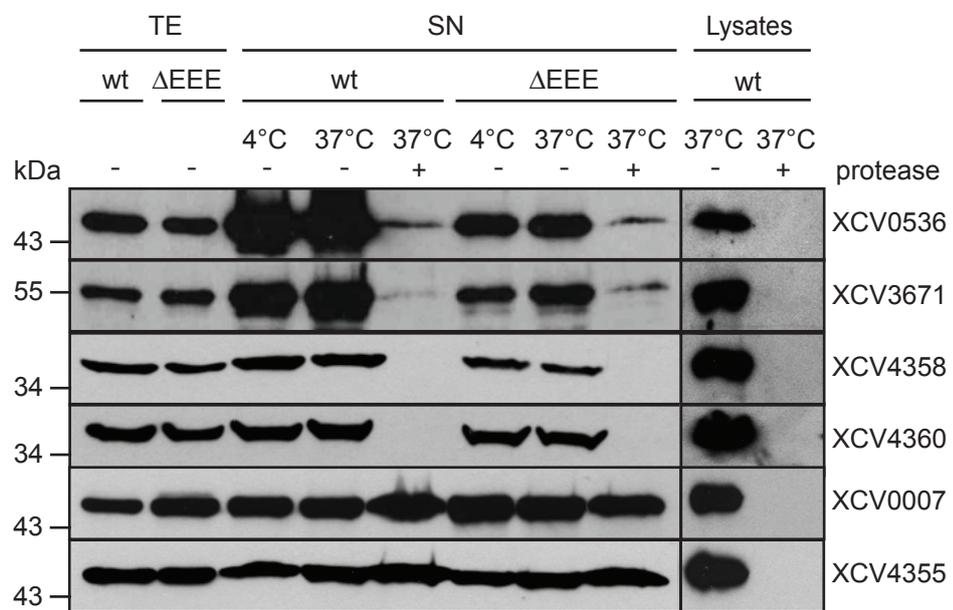


Fig. S5 XCV0007 and XCV4355 are protected from degradation by proteinase K when bacteria are incubated for one hour in NYG medium. Strains 85-10 (wt) and 85-10 Δ EEE (Δ EEE) containing expression constructs for XCV0536-c-Myc, XCV3671-c-Myc, XCV4358-c-Myc, XCV4360-c-Myc, XCV0007-c-Myc or XCV4355-c-Myc as indicated were incubated for one hour in NYG medium. Culture supernatants (SN) and bacterial lysates were incubated on ice or at 37°C in the presence (+) or absence (-) of proteinase K as indicated. Total cell extracts (TE), SN and lysates were analysed by immunoblotting, using c-Myc epitope-specific antibodies. For the detection of proteins in the culture supernatants, the blots were overexposed.

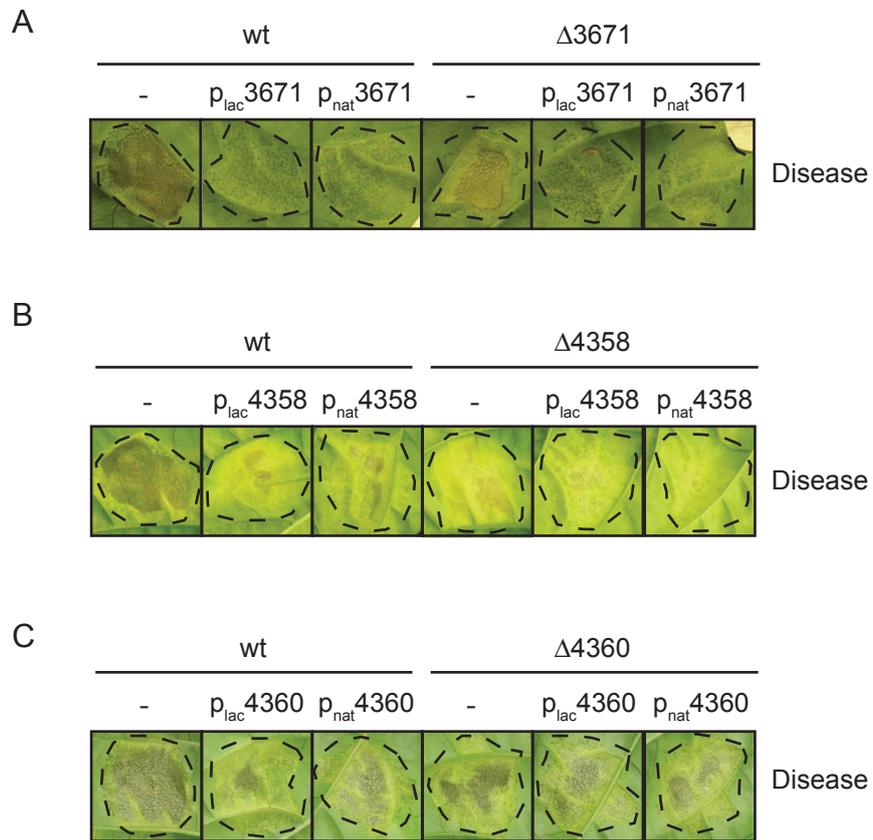


Fig. S6 Ectopic expression of XCV3671, XCV4358 or XCV4360 interferes with pathogenicity.

Strains 85-10 (wt), 85-10 Δ 3671 (Δ 3671) 85-10 Δ 4358 (Δ 4358) and 85-10 Δ 4360 (Δ 4360) without expression construct (-) or with expression constructs encoding XCV3671-c-Myc, XCV4358-c-Myc or XCV4360-c-Myc under control of the *lac* (p_{lac}) or the native XCV4361 promoter (p_{nat}) as indicated were inoculated into leaves of susceptible ECW pepper plants.

Disease symptoms were photographed 7 dpi. Dashed lines indicate the inoculated areas.

Table S1: Primers used in this study

Name	Sequence (5'-3') ^a
Primers for amplification of flanking regions of XCV4358	
XCV4358-FI-for	TTTGGTCTCT CGAC TCTATATCACCGAGTTCCG
XCV4358-FI-rev	TTTGGTCTCT GTTG GCATCCTCGATAAGTG
XCV4358-FII-for	TTTGGTCTCT CAAC CCGGGCTTCCTTCTCATTG
XCV4358-FII-rev	TTTGGTCTCT ATGG AACGCACGCCGTTGCCGATG
Primers for amplification of flanking regions of XCV4360	
XCV4360-FI-for	TTTGGTCTCT CGAC ATATCGGGGCCATTGTTGC
XCV4360-FI-rev	TTTGGTCTCT AGGC TCACCTCAGGACGATG
XCV4360-FII-for	TTTGGTCTCT GCCT CAATTATTGATGCGCGCC
XCV4360-FII-rev	TTTGGTCTCT ATGG GAGCCCGACATGGAAGTG
Primers for amplification of flanking regions of XCV3671	
XCV3671-FI-for	TTTGGTCTCT CGAC GTGATCTAGAGTTCTTCTAC
XCV3671-FI-rev	TTTGGTCTCT AACC GTGCCGGCCACGTG
XCV3671-FII-for	TTTGGTCTCT GGTT AACGGCCGATGTATCG
XCV3671-FII-rev	TTTGGTCTCT ATGG TCATTGGCGCGCCAGCAG
Primers for generation of expression constructs	
XCV0007-for	TTTGGTCTCT TATG AAAGTCTCAACCGCAAGC
XCV0007-rev	TTTGGTCTCT CACC CTTCAGGTTGATGATCTTC
XCV00024-for	TTTGGTCTCT TATG CGCGTAGCCGTCCTGTC
XCV00024-rev	TTTGGTCTCT GTTG GCCGGTTCGGTGGAC
XCV0027-for	TTTGGTCTCT TATG CCCCACAAGATCCTGTTGTGC
XCV0027-rev	TTTGGTCTCT CACC GCGTGGAGATTGGGCCTTG
XCV0536-for	TTTGGTCTCT TATG TTGCAGATCAACTCCCAGTC
XCV0536-prom-for	TTTGGTCTCT ATTC TGGAGACGAGCGAAC
XCV0536-rev	TTTGGTCTCT CACC GCGTCTGTTGTCCAAC
XCV0536-ATGrev	TTTGGTCTCT GTGC CATGAAGTGATGCTC
XCV0536-Secfor	TTTGGTCTCT GCAC CCGCGCGCGGT A C
XCV0583-for	TTTGGTCTCT TATG CTTACTCGCATCCGCTGTC
XCV0583-rev	TTTGGTCTCT CACC GCGATTGCTGGGAGAACG
XCV0673-for	TTTGGTCTCT TATG CCCATTTTCAGGACTGCAAG
XCV0670-rev	TTTGGTCTCT CACC ACCTGCAGCGCAGAAGCCGAAC
XCV0673-for	TTTGGTCTCT TATG AAGCGAGTTGCCGTGGGAC
XCV0673-rev	TTTGGTCTCT CACC CTTGCGTGCCGCCGCGCTG
XCV0730-for	TTTGGTCTCT TATG ACCTACACCGTTTCTTCGTG
XCV0730-rev	TTTGGTCTCT CACC GTCTGCCCAACGTCCCGGTG
XCV0805-for	TTTGGTCTCT TATG AGCAAGCGTTGGATCG

XCV0805-rev	TTTGGTCTCT CACC CCGAAGCGGCACGTTT
XCV0845-for	TTTGGTCTCT TATG ATGACGCGCTCTTTCTCTCAG
XCV0845-rev	TTTGGTCTCT CACC TGGCGCTGCCTGCGTCAG
XCV0889-for	TTTGGTCTCT TATG ACCATCCATCGCCTG
XCV0889-rev	TTTGGTCTCT CACC GCGTTGTTCCAATAC
XCV0961-for	TTTGGTCTCT TATG CCGTATCACTCAGTG
XCV0961rev	TTTGGTCTCT CACC ATACGATGCGCTGACAC
XCV0965-sigpep-for	TTTGGTCTCT TATG CAGACGGTGACGATCACGCCAC
XCV0965-rev	TTTGGTCTCT CACC GTTTCCGACCAGCGTGGCCAC
XCV1033-for	TTTGGTCTCT TATG TCCCTGATCGCCACTTTC
XCV1033-rev	TTTGGTCTCT CACC GCGGGCAGGATCTTGTTT
XCV1064-for_1	TTTGGTCTCT TATG AAGTCGAAGTCGATGTGCAC
XCV1064-rev_1	TTTGGTCTCT TAGC TGGTGCTCTCGTAG
XCV1064-for_2	TTTGGTCTCT GCTA CACCGTGACCGGCAACTTC
XCV1064-rev_2	TTTGGTCTCT CACC ACGGAAGGCCGCAATCG
XCV1075-for	TTTGGTCTCT TATG AGTGGTCCCGACGACG
XCV1075-rev	TTTGGTCTCT CACC GTCGTGTGCGTGCTGG
XCV1202-for	TTTGGTCTCT TATG GGGCTGGGCACGGCGAG
XCV1202-rev	TTTGGTCTCT CACC CTGCGCCAGTACCAC
XCV1311-for	TTTGGTCTCT TATG AAAATGCCACCCTGTTGTC
XCV1311-rev	TTTGGTCTCT CACC GCGCGCCTTGACAAATCG
XCV1335-for	TTTGGTCTCT TATG CAGTGGATTTCCCCGCGTTG
XCV1335-rev	TTTGGTCTCT CAAC TCGCAACTTGAGCATCAC
XCV1372-for	TTTGGTCTCT TATG AACCATCGCATCCGCAACCAG
XCV1372-rev	TTTGGTCTCT CACC GCCGCCGGCCTTCAAGGCCACGTAG
XCV2736-for	TTTGGTCTCT TATG ATGCGTTTGTCTGCTC
XCV2736-rev	TTTGGTCTCT CACC TTAAGTCCCTACCCGCAC
XCV2747-for	TTTGGTCTCT TATG GAACCCAGCGGC
XCV2747-rev	TTTGGTCTCT CACC TGCGGGCGCTCCTTG
XCV2993-for	TTTGGTCTCT TATG AACTCTCTTCGCAGGCGACCTC
XCV2993-rev	TTTGGTCTCT CACC GTGGCCCACCACGCGCATTTCG
XCV3283-for	TTTGGTCTCT TATG GTGTCCAGCGCATCCTCCATC
XCV3283-rev	TTTGGTCTCT CACC CCGCTGGAAGCGGCCGGTG
XCV3425-for	TTTGGTCTCT TATG CGTCGTCTCGCTGTTG
XCV3425-rev	TTTGGTCTCT CACC GCTCTCTCGGCGGTTG
XCV3639-for	TTTGGTCTCT TATG ATTAAGCCCTTGTTGCTG
XCV3639-rev	TTTGGTCTCT CACC CGGACGCACCTTGTTG
XCV3671-for	TTTGGTCTCT TATG ATCCAGAGTTCTTCTACGCAGGTC
XCV3671-rev	TTTGGTCTCT CACC TTGGCGCGCCAGCAGGCTCAC

XCV4096-for	TTT <u>GGTCTCT</u> <i>TATG</i> CGCATCCTGCTGTTGTCTG
XCV4096-rev	TTT <u>GGTCTCT</u> CACC CTTGCGCACCGCATCGCTCTTG
XCV4355-for	TTT <u>GGTCTCT</u> <i>TATC</i> GATCCAGTGCAATCG
XCV4355-rev	TTT <u>GGTCTCT</u> CACC CGTGCAGCGGTTGCGGTGG
XCV4358-for	TTT <u>GGTCTCT</u> <i>TATG</i> TTGAAACTCCGTTATCCGCTCAC
XCV4358-rev	TTT <u>GGTCTCT</u> CACC GGGCCTGCTGGCAACGTAG
XCV4358-sigpep-for	TTT <u>GGTCTCT</u> <i>TATG</i> GGGCCCATCGCTGCCGGCAAG
XCV4360-for	TTT <u>GGTCTCT</u> <i>TATG</i> CACAAGCGCGCAGTAG
XCV4360-rev	TTT <u>GGTCTCT</u> CACC TGGCGCAGGTGCACC
XCV4361-promfor	TTT <u>GGTCTCT</u> <i>AATC</i> AGGACAACGCGGTTCTGTG
XCV4361-promrev	TTT <u>GGTCTCT</u> CATA GCGCGTGGCCTATGCG
XCV4360-rev1	TTT <u>GGTCTCT</u> <i>GCGT</i> GGTTTCGAAGGCATG
XCV4360-for1	TTT <u>GGTCTCT</u> <i>ACGC</i> CGGAGGTGGCGATGTC
XCV4437-for	TTT <u>GGTCTCT</u> <i>TATG</i> CGCATCCATTCAAGACACATTC
XCV4437-rev	TTT <u>GGTCTCT</u> CACC GCGTGGCGCAAGCAGCTTG
XCC4118-for	TTT <u>GGTCTCT</u> <i>TATG</i> TCCACGCATGCATGCGCACTG
XCC4118-rev	TTT <u>GGTCTCT</u> CACC CGGCGCACGCACGCCAGGATGC
XCC0857-for	TTT <u>GGTCTCT</u> <i>TATG</i> AAGCCTTCCGTGTTGC
XCC0857-rev	TTT <u>GGTCTCT</u> CACC CTGCCCCACCAGCGTGGCGATG
XCC4115-for	TTT <u>GGTCTCT</u> <i>TATG</i> TTGCTGGGCTTGCTC
XCC4115-rev	TTT <u>GGTCTCT</u> CACC TCAGCCCTCCGCTTGCTTTC
xpsE-for	ATC <u>GAA</u> TCGTGAACGCGGTTGCC
xpsE-rev	GATGAGCTCCGCATCCTCCGTGAC
^a Restriction sites are underlined, 5'-overhangs generated after restriction with <i>Bsa</i> I are written in italics.	